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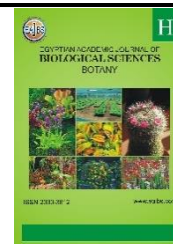
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**Multivariate Analyses of Physicochemical Factors Controlling Cyanobacteria Biodiversity in Al-Lith Thermal Springs, KSA.**

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**ABSTRACT**

Nowadays thermal springs are worldwide known sources of valuable Biobricks for biotechnological applications. The ecological analysis is a prerequisite to exploring thermophiles cyanobacteria that are a pervasive group in thermal springs globally. Therefore, this study aims to ecologically analyze cyanobacteria in Al-Lith area to extract growth factors that control prokaryotic primary producers and species biodiversity. Previously, there are four known thermal spring in Al-Lith, Saudi Arabia. This study discovered additional six novel springs that were never mentioned before. A seasonal sampling of cyanobacteria and physicochemical factors carried out from winter, 2015 to spring of 2016 in order to analyze factors controlling cyanobacteria species diversity. Temperature, total dissolved salt (TDS), pH, and dissolved oxygen and 17 chemical elements were investigated. A total of 34 species were identified in Al-Lith springs. Ghomygah thermal spring is the largest spring in Al-Lith region that is rich in high diversity of cyanobacteria. Oscillatoria and Chroococcus are dominant species and functionally tuned to all thermal habitat in Al-Lith springs regardless of the different growth conditions among them. The multivariate analysis helps resolve the complex relationships between physicochemical parameters and cyanobacteria biodiversity. Temperature is the pre-eminent factor determine the distribution of mat-forming cyanobacteria in spite of the difference in other factors. The remarkable increase in pH was observed with increases in cyanobacteria coverage. Therefore, pH is directly controlled by cyanobacteria. Thermal springs in Al-Lith grouped primarily based on physicochemical characteristics and secondly based on their geographical location. This work provides a comprehensive characterization of thermophiles native habitat that formulate a foundation for biotechnological deployment of cyanobacteria. Indeed, rational conservation of operative and healthy ecosystems are imperative to preserve these valuable resources.

**INTRODUCTION**

Cyanobacteria are remarkably pervasive and effective prokaryotic photoautotrophs that are essential primary producers in all aquatic habitats including extreme such as thermal springs (Hitzfeld *et al.*, 2000; Ward, 1998). Thermal springs are secluded habitats that are occurring as groups in globally detached areas. These microorganisms are extremophiles that are adapted to quite different growth conditions compared to the ambient milieu through which they would have to disperse (Papke *et al.*, 2003; Pentecost, 2003). Cyanobacteria are ample in most thermal waters where pH is greater than 6 and the temperature is below 72°

C. They are more abundant in hot springs with low mineral content that is inadequate to form deposits (Pentecost, 2014). Temperature is the pre-eminent factor determine the distribution of mat-forming cyanobacteria in spite of the difference in other factors (Roeselers *et al.*, 2006; Sompong *et al.*, 2005). Normally, species of the order Oscillatoriales are apparent in hot springs of temperature ranging from 40 to 66 °C (Pentecost, 2003; Pentecost *et al.*, 2003). Non- heterocystous, heterocystous and nitrogen-fixing species belonging to Oscillatoriales, Nostocales, and Stigonematales are the major components of the mat-forming cyanobacteria in thermal springs (Roeselers *et al.*, 2006; Sompong *et al.*, 2005). *Spirulina* sp. is widely distributed in hot springs worldwide including Saudi Arabia (Papke *et al.*, 2003; Pentecost *et al.*, 2003; Sompong *et al.*, 2005). The *Synechococcus* genus is a worldwide thermophile cyanobacterium that is naturally optimized to extreme temperatures (Dillon *et al.*, 2003; Miller *et al.*, 2007; Miller and Castenholz, 2000). *Synechococcus* species are adapted to a wide range of temperatures and chemically diverse springs (Ferris *et al.*, 1996). Diverse physicochemical stress factors are common in thermal springs (Papke *et al.*, 2003; Pentecost, 2003; Pentecost *et al.*, 2003; Purcell *et al.*, 2007) that elect specialized microorganisms in thermal springs adapted to these specific microhabitat variations (Ferris *et al.*, 1996; Miller *et al.*, 2007; Miller and Castenholz, 2000). Indeed, life in the hot springs is controlled by multi-stresses of complex interactions of the physicochemical factors (Miller *et al.*, 2002). Previously, only four Thermal spring recorded in Al-Lith, Saudi Arabia (Chandrasekharam *et al.*, 2015a, 2015b; Lashin *et al.*, 2015). Among these, Al Ain al Harrah in Ghomygah is the only known for public and have been used for recreational activities. In this study, ten thermal springs in Al-Lith region of Saudi Arabia investigated seasonally. Species diversity, physicochemical parameters were extracted by the state of the art tools. Multivariate analysis is employed to identify the interaction of factors that govern cyanobacteria biodiversity in thermal springs in Al-Lith.

## MATERIALS AND METHODS

**Study sites.** The study area is located in Al-Lith region, the mid-west coast of Saudia Arabia between latitude 20.298567 – 20.669361 and longitude 40.038778 – 40.700516 (Fig. 1). The investigated thermal springs are mainly distributed in four locations, wadi Sayaa, wadi Markub, Ghomygah, and Bin Halal villages.

**Physicochemical parameters.** Physical parameters analysis required on-site measurements to avoid unknown reactions of thermal waters and gather water properties in its virgin conditions. Therefore, EXO2 Multi-Parameter Water Quality Sonde (Yellow Spring, OH, USA). It is equipped with titanium sensors, bio-fouling protection with copper-alloy components and anti-fouling wipers. Sonde is connected with ROX dissolved oxygen probe, pH probe, conductivity/temperature probe, turbidity probe, and a chlorophyll/BGA probe. Each water quality datum is associated with a date, time, and GPS latitude and longitude coordinates, a Bluetooth wireless technology connects Sonde and handheld to the personal computer by KOR software. Sonde dropped in running thermal water and left for five minutes and three readings captured every three minutes. To analyze chemical parameters, subsurface water samples were collected at all sites in polyethylene bottles of one-liter capacity and in a pyrogenic free (Eaton *et al.*, 1998; Nienaber and Steinitz-Kannan, 2018). Three replicates were collected from different sites surrounding each sampling site at different distances. All chemical analyses of the macronutrient and micronutrients are analyzed by YSI kits used with YSI 9500 Photometer (Yellow Spring, OH, USA) according to the manufacturer protocols. Seasonal field visits to the study area started in winter 2015 till autumn 2016.



**Fig. 1.** Map of the study area in Al-Lith shows thermal springs investigated during this work, in Table (1) springs full names, short abbreviations and geographical locations are provided

### **Cyanobacteria Sampling:**

Samples collected seasonally, chemically fixed and freshly stored samples were both collected. Samples stored in screw cap glass bottles and partially filled to reduce inhibition of metabolic activities, then kept in an ice-box to avoid shocking the organisms due to a higher temperature.

### **Cyanobacteria Productivity and Species Abundance:**

For qualitative studies of phytoplankton species one liter of the collected water was fixed and concentrated using 1% of Lugol's solution (10 g of pure iodine, 2 g of potassium iodide, 200 ml of distilled water and 20 ml of glacial acetic acid; (Eaton *et al.*, 1998; Nienaber and Steinitz-Kannan, 2018)) samples were preserved in 500 ml glass bottles with Lugol's solution. At the laboratory, the samples were poured into a clean graduated glass cylinder of 500 ml capacity and 1% Lugol's solution was added, until the sample changed to faint tea color that facilitating sedimentation and staining of phytoplankton. The phytoplankton organisms were allowed to settle by gravitation for 5 days (Eaton *et al.*, 1998; Nienaber and Steinitz-Kannan, 2018). The supernatant was carefully siphoned off with a small plastic tube. The siphon was dipped into the cylinder, to about 3-4 cm from the bottom. The supernatant was siphoned off until the water surface was just a few millimeters above the siphon end until the samples were concentrated to about 50 ml. The reduced sub-sample was adjusted to 50 ml and stored into a dark glass bottle for microscopic examination. Qualitative analysis was carried out using the preserved as well as fresh samples.

### **Identification of Cyanobacteria Taxa:**

Species identification, as well as the standing crop, carried out using a phase-contrast microscope (Carl Zeiss, Jena). The characteristics of the species (length, width, thickness, and diameter) measured using an ocular and slide micrometer (Craticules, LTD, Tonbridge, Kent). Receipt materials deposited in Microbial Biotechnology Laboratory (MBL), Biology Department, Faculty of Science & Arts, and Jeddah University, Saudi Arabia. Photographs will take using an inverted microscope (OLYMPUS series 1 x 70 equipped with the SC35 camera (type 12) and a color video monitor Panasonic, Tc-1470Y. The main references used for identification of phytoplankton species were Desikachary (1959); Bourrely (1968); Prescott (1978); Anagnostidis and Komárek (1985, 1988, 1990); Komárek (1998); Van Vuuren, *et al.*, (2006); Komárek and Anagnostidis (2007); Komárek, *et al.*, 2013; Nienaber. and Steinitz-Kannan (2018).

## RESULTS

Rigorous searching for thermal springs in Al-Lith region reveals a total of ten thermal springs in the west coast region of the Red Sea, Saudi Arabia (Table 1). Of these, only four hot springs were previously described (Darakah, Markub, Ghomygah, and Mraag). Darakah Springs is located in wadi Sayaa north of Al-Lith (Fig 2 Dar-A-B). It is fenced by bricks to store thermal water that is utilized by grazing animals. Markub spring is uniquely characterized by orange coloration of sulfur deposits and high water flow rate (Fig. 2 Mrk-A-B). Ghomygah spring is well known locally as Al Ain Al Harrah (Fig 2 Gho-A-C). It is the official thermal spring for bathing and recreational activity in Al-Lith. Finally, Mraag spring located in Bin Halal village, with low water flow that flood 100 m<sup>2</sup> area with thermal water and form a shallow pond for cyanobacteria and algal growth (Fig 2 Mra-A-B). It is located in wadi bed that often covered with floodwater. However, the novel six springs described here for the first time are located in wadi Markub. Five in Massal named M1 to M5 (Table 1) and Raghaa. The novel five Massal springs are 50 – 100 meters apart (Fig. 2 M1-M5). Raghaa is characterized by low water flow that results in high cyanobacteria biomass compared to Markub and El-Massal. Interestingly, grazing animals commonly drink from the thermal water of wadi Markub springs. Therefore, animal waste is presumably a factor that may affect cyanobacteria.

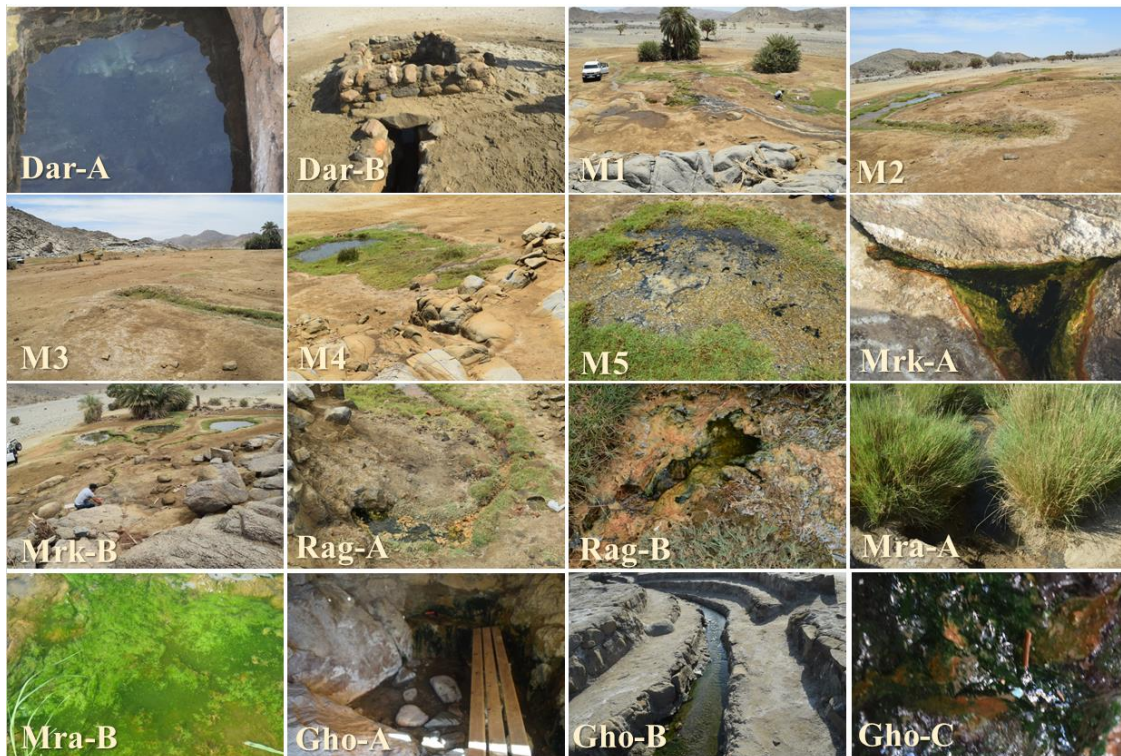
**Physical parameters:** physical parameters are key factors for the life of thermophiles in aquatic habitat particularly thermal springs. These factors sampled seasonally in order to understand their seasonal fluctuations and provide accurate data (Table 1). Temperature is varied in Al-Lith spring from 83 °C during the winter season in Ghomygah to as low as 24 °C in Darakah. The highest specific conductivity recorded in Markub (8537.37) and lowest was in Mraag (3479.38). High pH observed in both Darakah and Mraag (10.54 and 10.92 respectively). Mraag thermal spring is of moderate temperature and conductivity, but high pH that is favorable for vigor growth of cyanobacteria. Massal temperature varies from 35°C degrees to 45°C. However, SPCond, TDS and pH are similar in all thermal sources in Massal location during the study period.

**Chemical analysis:** Seventeen species of macro-, micro-, and toxic elements were analyzed (Table 2). In Darakah molybdenum, Bromine, chlorine and fluoride are among the lowest level in all Al-Lith springs. Chemical analysis of the sources of the Massal springs shows similar values. However, Manganese was below the detection limit in Massal springs. However, Copper, Zinc, Molybdenum, Nickel and Aluminum are highly concentrated in Massal springs. Chemically, Markub spring (Table 2) shows unique copper level compared to all springs examined that is 10 times the level in other springs and may be responsible for orange color deposit. Copper in Markub is 35 times compared to BG11 medium. Sulfate exist at a high level in all springs. The highest level of zinc found in Raghaa spring. Ghomygah spring shows the highest level of aluminum. Copper, zinc molybdenum are 6 to 10 times their concentrations in BG11 Medium. Sulfate and zinc in Mraag thermal water are 13 times their concentration in the BG11 medium.

filamentous mate-forming cyanobacteria. Where species of *Oscillatoria* are the backbone of the mats in Al-Lith thermal springs.

**Table 1.** Al-Lith springs investigated in this work, short names, latitude, longitude, and elevation of each spring are provided. Average seasonal values of the physical parameters measured by Sonde with seasonal variations in bold. SN: short names, Lat/long: Latitude/Longitude, Elev.: Elevation, Temp: Temperature, SPCond: Specific Conductivity, TDS: Total dissolved salt, ODO: Dissolved oxygen, BGA: Blue-green algae, Baro: Pressure.

Thermal spring	SN	Location Lat/long	Elev. m	Temp °C	SPCond $\mu\text{S}/\text{cm}$	TDS mg/L	pH	ODO mg/L	BGA $\mu\text{g}/\text{L}$	Baro mmHg
Darakah	Dar	20.669361	144	30.10	6,139.70	3,710.75	10.92	4.48	0.52	811.87
		40.038778		<b>6.31</b>	<b>2,273.74</b>	<b>1,100.09</b>	<b>4.52</b>	<b>1.28</b>	<b>0.60</b>	<b>121.27</b>
Massal 1	M1	20.520389	110	43.40	7,276.28	3,819.17	7.52	2.19	1.82	749.03
		40.141722		<b>0.79</b>	<b>1,165.27</b>	<b>31.63</b>	<b>0.62</b>	<b>3.33</b>	<b>1.71</b>	<b>2.94</b>
Massal 2	M2	20.520636	112	41.80	6,847.53	3,883.33	7.89	3.50	24.48	748.15
		40.143009		<b>2.36</b>	<b>1,336.05</b>	<b>65.53</b>	<b>0.14</b>	<b>2.49</b>	<b>30.71</b>	<b>3.28</b>
Massal 3	M3	20.520190	112	40.43	6,831.59	3,810.75	8.05	2.85	14.44	748.07
		40.142749		<b>2.67</b>	<b>1,360.70</b>	<b>6.01</b>	<b>0.39</b>	<b>3.53</b>	<b>18.87</b>	<b>3.35</b>
Massal 4	M4	20.520232	111	40.69	6,845.53	3,839.83	8.09	2.05	12.58	748.08
		40.142920		<b>1.34</b>	<b>1,322.89</b>	<b>2.59</b>	<b>0.43</b>	<b>2.65</b>	<b>15.79</b>	<b>3.29</b>
Massal 5	M5	20.520596	110	39.34	6,727.72	3,861.00	7.60	3.47	8.50	748.02
		40.142967		<b>0.81</b>	<b>1,184.99</b>	<b>46.20</b>	<b>1.12</b>	<b>2.59</b>	<b>5.39</b>	<b>3.37</b>
Markub	Mrk	20.525472	129	53.75	8,537.37	3,908.88	7.62	0.54	2.28	747.00
		40.155861		<b>2.09</b>	<b>1,534.71</b>	<b>105.65</b>	<b>0.86</b>	<b>0.25</b>	<b>3.07</b>	<b>2.49</b>
Raghaa	Rag	20.520472	109	41.08	7,291.94	3,923.44	8.79	2.45	4.17	746.24
		40.140369		<b>2.98</b>	<b>1,029.14</b>	<b>68.41</b>	<b>0.97</b>	<b>2.43</b>	<b>6.42</b>	<b>1.26</b>
Ghomygah	Gho	20.461369	165	69.08	6,532.95	3,630.50	8.57	12.60	10.49	745.84
		40.471283		<b>13.90</b>	<b>2,200.26</b>	<b>1113.28</b>	<b>1.25</b>	<b>23.01</b>	<b>20.93</b>	<b>2.56</b>
Mraag	Mra	20.298567	178	37.62	3,479.38	1,844.78	10.54	1.81	0.71	746.57
		40.700516		<b>12.52</b>	<b>1,105.16</b>	<b>620.78</b>	<b>5.43</b>	<b>2.40</b>	<b>0.63</b>	<b>3.51</b>



**Fig. 2.** High-resolution images of Al-Lith thermal springs named by their short names that are listed in Table 1. Images provide detailed characteristics of the thermal springs and their cyanobacteria communities.

Species diversity in Al-Lith springs: A total of 34 species of cyanobacteria were recorded in Al-Lith springs (Table 3). Ghomygah shows the highest diversity of cyanobacteria by 34 species. However, Massal 3 was characterized by a low diversity of 18 species. But, Massal 4 supported 31 species. Indeed, Massal site provides an interesting opportunity to study species composition and diversity in different thermal sources with close proximity. In Mraag, thermal water mixed with the flood that forms a pond of moderate-temperature that allows *Nostoc*, *Oscillatoria* and even the green algae *Spirogyra* to bloom. Sixteen species are highly frequent (90 %) in Al-Lith springs. Of these, only four species belong to three unicellular genera. However, 75 % of the highly frequent species are filamentous mate-forming cyanobacteria. Where species of *Oscillatoria* are the backbone of the mats in Al-Lith thermal springs.

**Table 2.** Chemical analysis of macro-elements, microelements and toxic species of the thermal water of Al-Lith springs. M: Mean value of three analyses, SD: Standard deviations. M1 –M5: five Massal springs.

Chemical species	Darakah		M1 - M5		Raghaa		Markub		Ghomygah		Mraag	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
<b>Macroelements</b>												
Phosphate ug/L	215	35	330	14	195	7	370	14	205	92	285	21
Nitrate ug/L	1	0	1	0	1	0	1	0	1	0	1	0
Calcium ug/L	908	11	635	21	860	57	878	25	516	133	402	37
Potassium mg/L	7	1	6	0	7	1	7	1	5	1	6	0
Sulfate mg/L	360	14	315	7	335	35	345	21	375	21	365	21
Magnesium mg/L	0	0	2	0	0	0	0	0	0	0	0	0
<b>Microelements</b>												
Manganese ug/L	42	9	0	0	59	3	58	4	2	0	42	4
Molybdenum ug/L	500	307	1563	152	1063	617	1017	306	1573	172	630	228
Copper ug/L	0	0	400	24	160	10	700	43	120	7	120	7
Zinc ug/L	607	60	833	76	1567	126	667	21	500	50	650	50
Nickel ug/L	177	25	133	58	167	115	417	76	160	53	117	126
<b>Toxic elements</b>												
Nitrite ug/L	4	1	2	0	3	3	7	1	7	1	0	0
Sulfite mg/L	6	3	7	1	7	1	6	0	11	1	7	1
Silica mg/L	54	2	80	12	80	2	80	4	93	9	87	5
Aluminum ug/L	70	14	22	3	54	8	90	14	140	28	80	0
Bromine ug/L	385	33	390	212	510	14	560	0	540	70	550	71

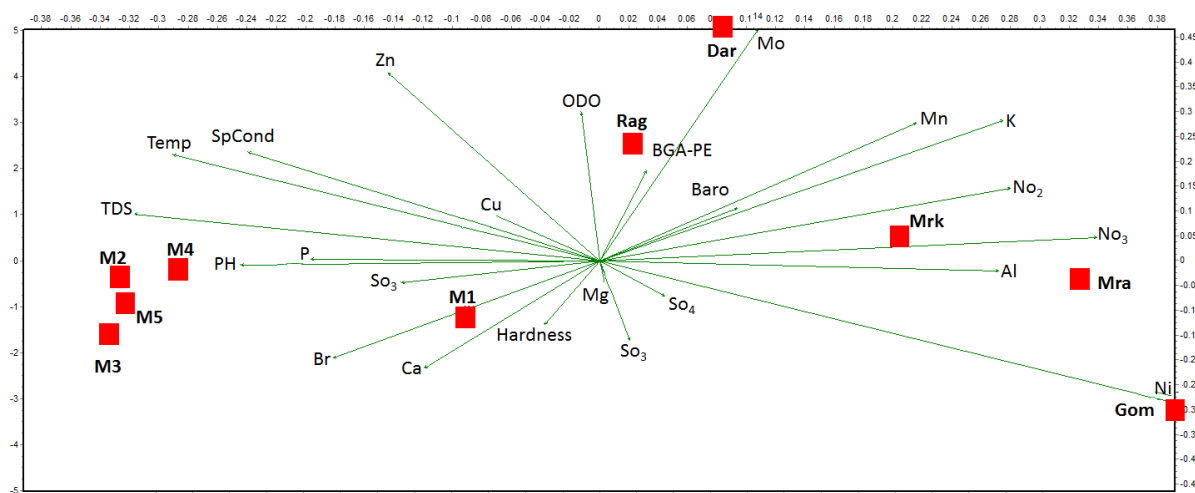
Multivariate analysis: To understand the factors that govern species diversity in Al-Lith springs, we used multivariate statistical analyses to examine the complex interactions between the physicochemical characteristics (Fig. 3 & 4). Principle components analysis (PCA) plot ordination of physicochemical factors (Fig. 3) of Al-Lith thermal springs based on the physicochemical parameters values represented by vector arrows, arrow length and direction is indicative of the ecological relativity to which the factor control species biodiversity. Bottom-up hierarchical clustering (agglomerative) based on complete linkage (Fig. 4A-C) provides a simple understanding of the relationships between physical and chemical factors in Al-Lith springs. Multivariate of the physical factors generates two clusters. Cluster 1 shows temperature as a leading factor that is highly correlated with

dissolved oxygen (ODO). Within cluster 1, blue-green algae measured as phycoerythrin (PE) form a sub-cluster with pH that is indicative of a strong correlation between them. However, SPCond and TDS form a separate group as cluster 2. Cluster analysis of the chemical species (Fig. 4B) ordered macronutrient species in the upper part and micronutrient and toxic chemical species sorted out in the lower part. Cluster analysis for Al-Lith springs based on the physicochemical and species diversity data generates an interesting dendrogram analysis (Fig. 4C). Ghomygah spring found in one separate cluster. However, all the other springs grouped into one major cluster that easily divided into two groups. The first Sub-cluster contains Markub and Raghaa and Mraag. However, Darakah forms a group with Massal springs. The high similarity of Massal springs implies that these sources may be connected to the underground layers. Indeed, Al-Lith springs grouping is probably based on the temperature that is mainly the driving factors that shape cyanobacteria species biodiversity in Al-Lith thermal springs (Fig. 4C).

**Table 3.** Cyanobacteria species recorded in Al-Lith thermal springs, frequency (Freq.) of each species and a total number of species recorded in each spring are calculated.

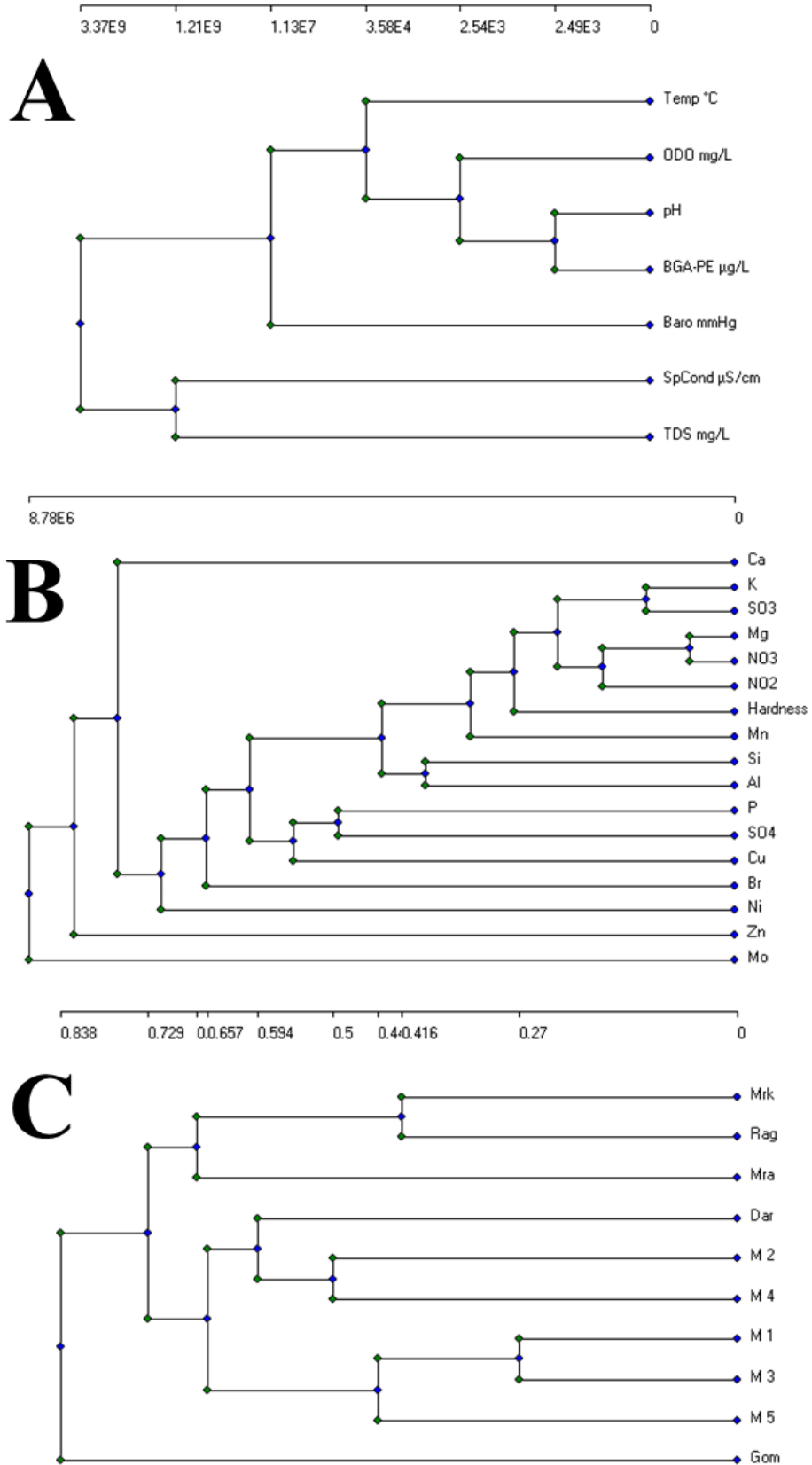
Species name	Freq.	Al-Lith hot springs									
		Gom	Mra	M 4	M 1	Mrk	Dar	Rag	M 2	M 5	M 3
1. <i>Chroococcus yellowstonensis</i> Copeland	10	+	+	+	+	+	+	+	+	+	+
2. <i>Oscillatoria princeps</i> Vaucher and Gomont	10	+	+	+	+	+	+	+	+	+	+
3. <i>Cyanothece</i> sp.	10	+	+	+	+	+	+	+	+	+	+
4. <i>Oscillatoria filiformis</i> J.Copeland	10	+	+	+	+	+	+	+	+	+	+
5. <i>Schizothrix calcicola</i> Gomont	10	+	+	+	+	+	+	+	+	+	+
6. <i>Spirulina subsalsa</i> Oersted ex Gomont	10	+	+	+	+	+	+	+	+	+	+
7. <i>Thermosynechococcus elongatus</i> Robert E. Brucoleri	10	+	+	+	+	+	+	+	+	+	+
8. <i>Oscillatoria amphibia</i>	10	+	+	+	+	+	+	+	+	+	+
9. <i>Oscillatoria rubescens</i>	10	+	+	+	+	+	+	+	+	+	+
10. <i>Oscillatoria proboscidea</i> Gomont	9	+	+	-	+	+	+	+	+	+	+
11. <i>Oscillatoria willei</i> Gardn.	9	+	+	-	+	+	+	+	+	+	+
12. <i>Phormidium cebennese</i> Gomont S	9	+	+	+	+	+	+	+	-	+	+
13. <i>Chroococcus ansuyensis</i> Vasishta	9	+	+	+	+	+	+	+	+	+	-
14. <i>Anabaena variabilis</i> Komárek & Anagnostidis	9	+	+	+	+	+	+	+	+	+	-
15. <i>Mastigocladus laminosus</i> Cohn ex Kirchner	9	+	+	+	+	+	+	-	+	+	+
16. <i>Oscillatoria rosea</i>	9	+	+	+	+	+	+	+	+	+	-
17. <i>Lyngbya aerugino-coerulea</i> (Kutz.) Gomont	8	+	+	+	+	+	+	+	-	+	-
18. <i>Gloeocapsa gelatinosa</i> Kutzing	8	+	+	+	+	+	+	-	+	+	-
19. <i>Microcoleus vaginatus</i> Gomont ex Gomont	8	+	+	+	+	+	+	-	+	-	+
20. <i>Oscillatoria cortiana</i> Gomont	8	+	+	+	+	+	+	-	+	+	-
21. <i>Phormidium terebriformis</i> (C. Agardh ex Gomont)	8	+	+	+	+	+	-	-	+	+	+
22. <i>Calothrix thermalis</i> Hasngirg ex Bornet & Flahault	8	+	+	+	+	+	+	-	-	-	+
23. <i>Oscillatoria limosa</i> (Roth) C. Agardh	7	+	+	+	+	-	+	+	+	-	-
24. <i>Merismopedia tenuissima</i> Lemmermann	7	+	+	+	+	+	+	-	-	-	+
25. <i>Synechococcus bigranulatus</i> Skuja	6	+	+	+	+	+	+	-	-	-	-
26. <i>Aphanocapsa grevillei</i> (Berkeley) Rabenhorst	6	+	+	+	-	+	+	-	-	+	-
27. <i>Plectonema notatum</i> Anagnostidis & Komárek	6	+	+	+	+	-	-	+	-	-	+
28. <i>Rivularia</i> sp.	5	+	+	+	-	+	-	-	+	-	-
29. <i>Anacystis</i> sp	5	+	+	+	+	-	-	+	-	-	-
30. <i>Cyanothece aeruginosus</i>	5	+	-	-	+	+	+	+	-	-	-
31. <i>Lyngbya nigra</i> (Gomont) Anagnostidis & Komárek	4	+	+	+	+	-	-	-	-	-	-
32. <i>Phormidium tenue</i> Gomont S	4	+	+	+	-	+	-	-	-	-	-
33. <i>Lyngbya diqueti</i> (Kutz.) Gomont	4	+	+	+	-	-	+	-	-	-	-
34. <i>Scytonema leptobasis</i> Ghose	3	+	-	+	-	-	-	+	-	-	-
Total species present		34	32	31	29	28	27	22	21	21	18



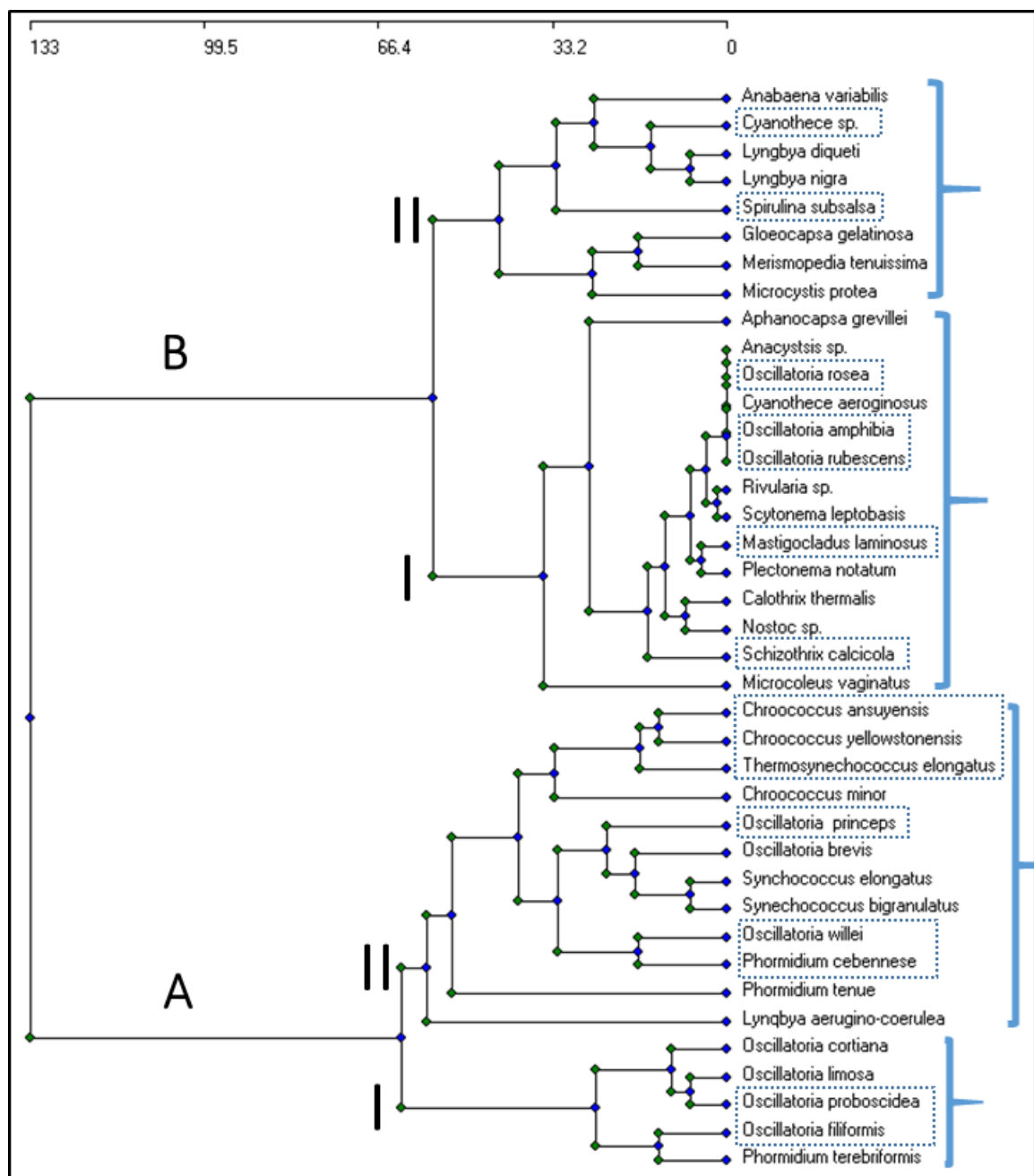


**Fig. 3.** PCA ordination plot of Al-Lith thermal springs based on the physicochemical parameters values represented by vector arrows, arrow length and direction is indicative of the ecological relativity to which the factor control species biodiversity. Al-Lith thermal springs are marked by red squares and named according to their short names listed in Table (1). Physical factors abbreviations are as follow Temp: Temperature, SPCond: Specific Conductivity, TDS: Total dissolved salt, ODO: Dissolved oxygen, BGA: Blue green algae, Baro: Pressure. Standard chemical species (Ca: Calcium, K: potassium, PO<sub>4</sub>: Phosphate, NO<sub>3</sub>: Nitrate, SO<sub>4</sub>: Sulfate, Mg: Magnesium, Mn: Manganese, Cu: Cupper, Zn: Zinc, Mo: Molybdenum, Ni: Nickel, NO<sub>2</sub>: Nitrite, Si: Silicon, SO<sub>3</sub>: Sulfite, Br: Barium, Al: Aluminum).

Species dendrogram analysis: Agglomerative cluster analysis based on the average linkage of species compositions in Al-Lith springs reveal two groups and four subgroups (Fig. 5). Clusters are characterized by similar life forms (unicellular versus filamentous) and physiologically relevant species (nitrogen-fixing versus non-nitrogen fixing). Furthermore, cluster A composed of species of the order Chroococcales that are associated with mat-forming and highly adapted thermophiles cyanobacteria species of the genus *Oscillatoriales*. Highly frequent species (90 - 100 % frequency; Table 3) were scattered in all clusters. Interestingly, seven species of *Oscillatoria* genus are among the highly frequent species (Table 3). These species are scattered in three sub-clusters (AI, AII and BI; Fig. 5). Indeed, *Oscillatoria* species are apparently the major component of cyanobacteria mats in the thermal springs in Al-Lith area.



**Fig. 4.** Cluster analysis of the physical parameters (A), chemical species (B) and thermal springs (C) in Al-Lith area, Saudi Arabia.



**Fig. 5.** Species agglomerative cluster analysis based on the average linkage of species generated by Twinspan program in Al-Lith thermal springs. Dendrogram forms two groups (A & B), each split into two subgroups (I & II). Highly frequent species (above 90%) are framed by the dotted quadrangle.

## DISCUSSION

Al-Lith region is rich with 10 diverse thermal springs. Physicochemical variables show high variations between them. Cyanobacteria are abundant in all Al-Lith thermal springs where pH is greater than 7.5 and the temperature is below 70° C that is common in other thermal springs in a different part of the world (Pentecost, 2003; Pentecost *et al.*, 2003; Purcell *et al.*, 2007; Roeselers *et al.*, 2006). Species composition of cyanobacteria in Al-Lith springs is correlated with the physicochemical variables. Temperature seems to be the pre-eminent factor that shapes species diversity. This observation is similar to thermal springs

in temperate regions (Miller and Castenholz, 2000) as well as in tropical regions (Purcell *et al.*, 2007; Roeselers *et al.*, 2006). However, the temperature is governing species diversity, pH is regulated by species abundance that is evident with the formation of cluster fusing BGA-PE with pH (Fig. 4A). Life in the hot springs is controlled by multi-stresses due to the complex interactions of the physicochemical factors (Miller and Castenholz, 2000). In spite of the variations in other environmental variables among these springs. It is still difficult to identify the chemical factors that influence the species composition, distribution, and abundance in spring due to the complex interactions in these thermal ecosystems (Miller and Castenholz, 2000).

The ecological gradient of the physicochemical parameters in Al-Lith thermal springs is mainly the cause of reasonable biodiversity. Interestingly, all species of cyanobacteria recorded in this study are present in Ghomygah springs. Markub spring with a unique chemical composition of sulfur, copper, zinc, and molybdenum is occupied by *Oscillatoria* as the dominant mat-forming cyanobacteria. This is maybe due to that species of *Oscillatoria* genus are adapted to colonize anaerobic, sulfide-containing habitats (Dillon *et al.*, 2007; Dillon and Castenholz, 2003). Because, at night time of the day, *Oscillatoria* ferment glycogen formed from the daytime photosynthetic activity (Fishbain *et al.*, 2003; Teske, 2010). It is not only *Oscillatoria* but also *Phormidium* and *Lyngbya* seem to adapt to Al-Lith thermal springs habitat, they are filaments taxa that are successful mat-forming cyanobacteria. Indeed, species of the order Oscillatoriales are apparent in hot springs of temperature ranging from 40 to 66 °C (Pentecost, 2014, 2003; Pentecost *et al.*, 2003) and their exopolysaccharide sheath is probably the backbone of the microbial mat developed (Abdelwahab and Amin, 2018). It has been reported that most conspicuous species of cyanobacteria in hot springs of temperature ranging from 40 to 66 °C belong to the order Oscillatoriales (Abdelwahab and Amin, 2017) that agrees with *Oscillatoria* as the prevailing genus in Al-Lith springs. Furthermore, the occurrence of filamentous cyanobacteria belonging to the Oscillatoriales in sulfur-rich environments of Al-Lith springs is well documented (Mohamed, 2008; Spring *et al.*, 2003). Non- heterocystous, heterocystous and nitrogen-fixing species belonging to Oscillatoriales, Nostocales and Stigonematales are the major components of the mat-forming cyanobacteria in Al-Lith thermal springs, because they are well adapted to thermal habitat (Purcell *et al.*, 2007; Roeselers *et al.*, 2007).

Interestingly, *Chroococcus* and *Synechococcus* are planktonic forms, however, they are establishing themselves in the filamentous mats. Therefore, filamentous mat-forming cyanobacteria is a crucial habitat for unicellular species in thermal springs. The presence of *Synechococcus* confirms the fact that *Synechococcus* developed a unique adaptation to extreme temperatures (Miller and Castenholz, 2000). *Synechococcus* species are found not only in a wide range of temperatures but also in chemically diverse hot springs (Ramsing *et al.*, 1997).

Indeed, multivariate analysis was successful to explain the complexity of physicochemical factors in Al-Lith springs. Physical factors are major contributors to shaping cyanobacteria species biodiversity. However, chemical characteristics of thermal water did not significantly play a role in the life of thermophile cyanobacteria in Al-Lith springs. Thermal springs located in close proximity were clustered together like Markub and Raghaa and Massal springs. Unique thermal spring-like Ghomygah is easily separated by PCA analysis. This study provides detailed ecological analyses to a diverse group of natural resources of thermal habitat. Providentially, anthropogenic impact but did not decrease cyanobacteria biodiversity especially in Ghomygah springs. It has the highest recorded species in Al-Lith area. Indeed, Ghomygah is unique spring in Al-Lith that requires special management and conservation programs.

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